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Preactivated thiolated nanoparticles: A novel mucoadhesive dosage form

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ABSTRACT

Within this study a novel form of mucoadhesive nanoparticles (NPs) exhibiting a prolonged residence time on mucosal tissues was developed.

In order to achieve this goal a new thiomer was synthesized by the covalent attachment of the amino acid L-cysteine ethyl ester to poly(acrylic acid) (100 kDa). The free thiol groups were in the following preactivated with the aromatic thiol bearing ligand 2-mercaptonicotinic acid (2-MNA) and the amount of coupled L-cysteine ethyl ester as well as the amount of attached 2-MNA was determined. Based on this, preactivated thiomer NPs were prepared by ionic gelation with polyethylenimine (PEI). The resulting NPs were characterized regarding size and zeta potential. Furthermore their mucoadhesive properties were investigated via rheological measurements with porcine intestinal mucus and via determination of the particles' mucosal residence time.

Results showed that 1666.74 μmol L-cysteine ethyl ester and 603.07 μmol 2-MNA could be attached per gram polymer. NPs were in a size range of 112.67–252.84 nm exhibiting a zeta potential of −29 mV. Thiolated NPs only led to a 2-fold increase in mucus viscosity whereas preactivated NPs showed a 6-fold higher mucus viscosity than unmodified NPs. The mucosal residence time of thiolated NPs was 1.6-fold prolonged and that of preactivated NPs even 4.4-fold higher compared to unmodified particles.

Accordingly, preactivated thiolated NPs providing a prolonged residence time on mucosal membranes could be a promising dosage form for various applications.

1. Introduction

Since the early 1980s, mucoadhesive drug delivery systems have gained increasing importance in the field of pharmaceutical technology (Boddupalli et al., 2010). They are characterized by a prolonged residence time on mucosal tissues at the application or absorption site due to their ability to interact with the mucus gel layer. In addition an intimate contact between the dosage form and the underlying absorption membrane can be achieved improving therapeutic drug performance. A further advantage is the localization of the dosage form at a specific target site. More recently, the concept of mucoadhesion was further developed by the design of mucoadhesive NPs providing additional benefits such as controlled drug release, improved drug uptake and drug targeting (Zambito et al., 2013). At the same time, mucoadhesive NPs are able to interact with different mucosal membranes such as nasal, ocular, buccal, intravesical, vaginal and intestinal mucosa.

One promising strategy in order to obtain mucoadhesive NPs was the development of NPs based on thiolated polymers or so called thiomers. These NPs are able to form disulfide bonds with the mucus gel layer resulting in a considerable superiority in mucoadhesive properties over unmodified particles (Barthelmes et al., 2013). However, free thiol groups are comparatively unstable in solutions as they are oxidized at pH ≥ 5 (Hauptstein and Bernkop-Schnurch, 2012). In order to overcome this shortcoming, protective substructures were introduced. It was shown that coupling of 2-MNA via disulfide bond formation can prevent thiol oxidation leading to even further improved mucoadhesive properties (Iqbal et al., 2012). What is already well established for polymers was not researched in the field of nanoparticulate systems yet as the concept of preactivation was not transferred to nanoparticles so far (Hauptstein and Bernkop-Schnurch, 2012).

It is therefore the aim of the study to prepare preactivated thiomer NPs and to evaluate their mucoadhesive properties. In

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order to achieve this goal, a novel thiolated polymer was synthesized by attachment of cysteine ethyl ester to polyacrylic acid. This comparatively more hydrophobic esterified amino acid ligand was chosen, on the one hand, in order to additionally improve the mucoadhesive properties of the resulting NPs via lipophilic interactions with fatty acid substructures of the mucus and on the other hand to avoid undesired side reactions of the carboxylic acid groups during the coupling reaction (Smart, 2005). After preactivation with 2-MNA as illustrated in Fig. 1, NPs were formed via ionic gelation with PEI and characterized regarding particle size, zeta potential, polydispersity index and cytotoxicity. Furthermore, their mucoadhesive properties were investigated and compared to thiolated and unmodified NPs with two different evaluation methods.

2. Materials and Methods

2.1. Materials

2-Mercaptonicotinic acid (2-MNA) was purchased from ABCR GmbH & Co. KG, Karlsruhe, Germany. Polyethylenimine (60 kDa, Mwt 60.0 Da, 50% (w/w) aqueous solution) was purchased from Alfa Aesar GmbH & Co., KG, Karlsruhe, Germany. Acetonitrile was obtained from VWR chemicals, Radnor, PA, USA. D-(+)-Trehalose dihydrate was purchased from TCI chemicals, Eschborn, Germany. Poly(acrylic acid) (100 kDa Mwt, 35% (w/w) aqueous solution) and all other chemicals were purchased from Sigma–Aldrich, Vienna, Austria.

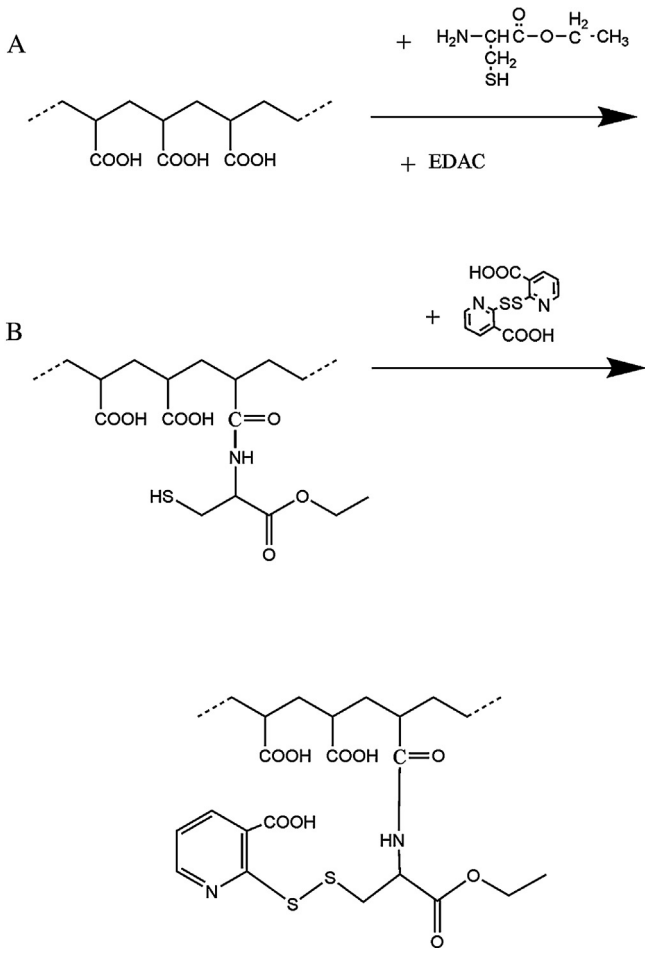

Fig. 1. Synthetic pathway for the generation of PAA-cysteine ethyl ester (A) and PAA-cysteine ethyl ester-2MNA (B).

2.2. Methods

2.2.1. Synthesis of thiolated PAA

Thiolated PAA was synthesized according to a previously described method with slight modifications (Bonengel et al., 2014). In brief, 1 g of PAA was dissolved in 150 ml demineralized water and the pH was adjusted to 5.8 using 5 M NaOH. Afterwards, EDAC dissolved in distilled water was added to the polymer solution in a final concentration of 200 mM in order to activate carboxylic acid moieties. After an incubation time of 15 min under stirring, 0.5 g of L-cysteine ethyl ester dissolved in 25 ml distilled water was added. The pH was readjusted to 5.8 and the reaction mixture was stirred at room temperature for 6 h. The thiolated polymer was finally purified by exhaustive dialysis at 10 °C and lyophilized under reduced pressure (Christ Gamma 1–16 LSC Freeze dryer). The amount of free thiol groups immobilized on the polymeric backbone was quantified photometrically with a microplate reader (Tecan infinite, M200 spectrometer, Grödig, Austria) using Ellman's reagent as previously described by our research group (Bernkop-Schnurch et al., 1999). The total amount of attached L-cysteine ethyl ester was determined by performing the same quantitative test after the reduction with $NaBH_4$ and adding Ellman's reagent. Furthermore, a test with 2,4,6-trinitrobenzenesulfonic acid (TNBS) was performed in order to quantify unbound amino acid by detecting primary amino groups.

2.2.2. Synthesis of preactivated thiolated PAA

The preactivated form of thiolated PAA was obtained by covalent attachment of 2-mercaptonicotinic acid via disulfide bond formation between the polymeric thiol groups and the thiol group of the aromatic ligand. The synthesis was performed according to a previously described method (Bonengel et al., 2014). First, the dimeric form of 2-mercaptonicotinic acid was prepared by oxidation with hydrogen peroxide. In a second step, 500 mg of thiolated polyacrylic acid was dissolved in 100 ml demineralized water under stirring at room temperature. Then, 500 mg of 2,2′-dithiodinicotinic acid was added to the polymer solution and the pH was adjusted to 8 using 5 M NaOH. The reaction mixture was finally stirred at room temperature for six hours. The resulting conjugate was exhaustively dialyzed at 10 °C and freeze dried. The amount of attached 2-MNA was quantified using a spectrophotometric method which was previously described by our research group (Iqbal et al., 2012).

2.2.3. Preparation of fluorescently labelled NPs

Three different kinds of particle formulations were prepared: NPs comprising unmodified PAA, thiolated PAA and preactivated PAA. This was accomplished by formation of poly-ion complexes between the particular polyacrylic acid derivative and PEI. Therefore, PEI, unmodified PAA, thiolated PAA and preactivated PAA were dissolved in Tris buffer (20 mM, pH 7.5) in a final concentration of 0.5% (m/v). Afterwards the PEI solution was added drop by drop under continuous stirring (350 rpm) to a solution of the particular polyacrylic acid derivative in a volume ratio of 1:2.5. This corresponds to a molar ratio of interacting functions (COOH: NH) of 1.5:1 for PAA, 1.1:1 for thiolated PAA and 1:1 for preactivated PAA. After 30 min a freshly prepared 0.05% (m/v) solution of fluorescein diacetate (FDA) dissolved in acetonitrile was added to the nanoparticle suspension in a volume ratio of 1:1. Then, the suspensions were incubated at 10 °C in the dark under continuous shaking (Thermomixer) for two hours. The nanoparticle suspensions were centrifuged three times (4300 rpm, 20 min) for purification. Thereby, 2% trehalose was added in order to avoid particle aggregation. Pellets were washed with a 50% (v/v) acetonitrile solution after the first centrifugation, whereas distilled water was used after the second and third centrifugation.